The American Journal of Human Genetics, Volume *90*

Supplemental Data

Mutations in *NSUN2* Cause Autosomal-Recessive Intellectual Disability

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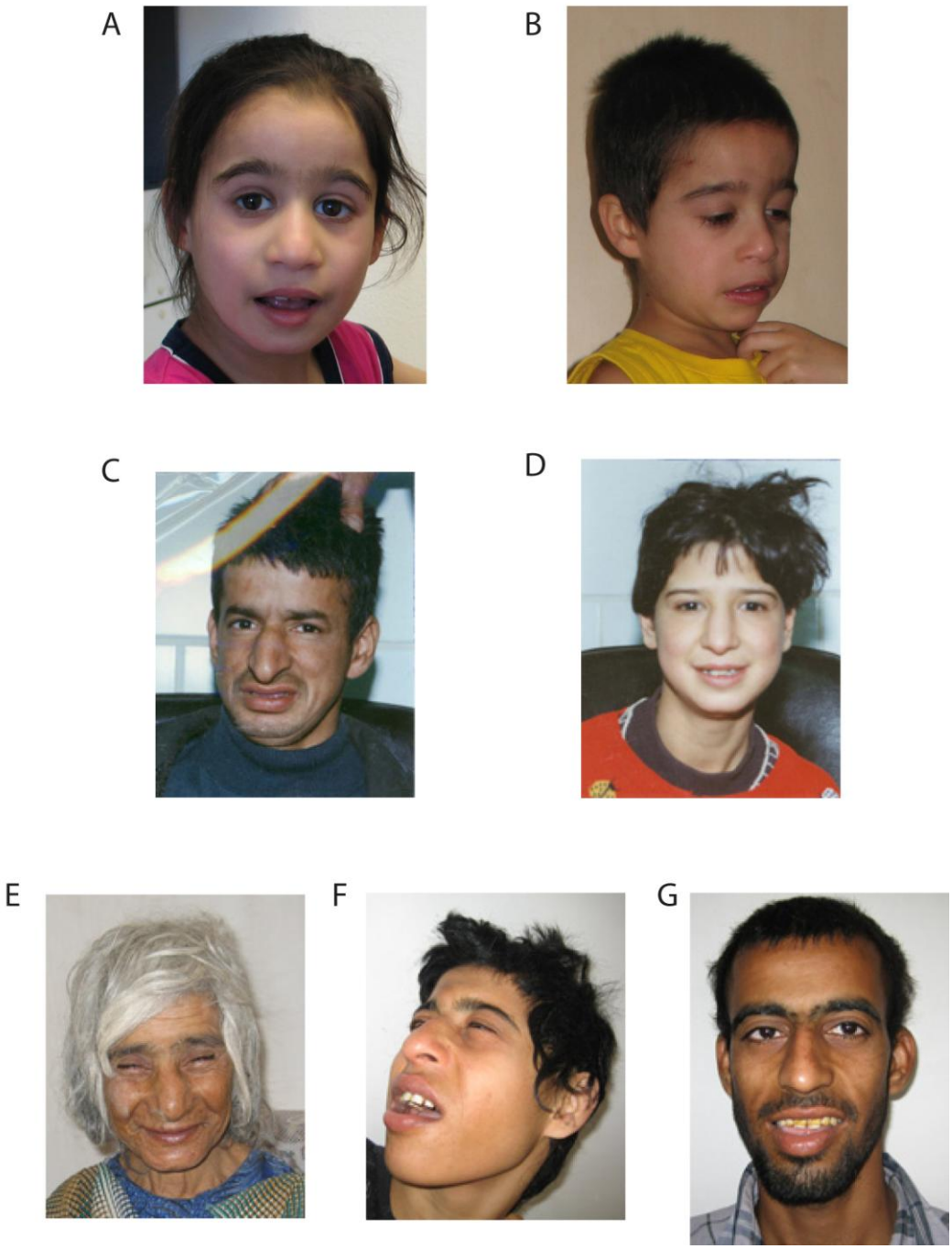

Figure S1. Facial Dysmorphism in Affected Individuals Consisting of Long and Narrow Face, Bushy Eyebrows with Synophrys, Hypotelorism, Large Nose with Long Columella and Hypoplastic Alae Nasi, Short Philtrum and Full Upper Lip

(A) Individual IV:5 of family G-013.

(B) Individual IV:6 of family G-013.

(C) Individual IV:6 of family M-192

(D) Individual IV:5 of family M-192.

(E) Individual V:1 of family M-314.

(F) Individual VI:4 of family M-314.

(G) Individual VI:7 of family M-314.

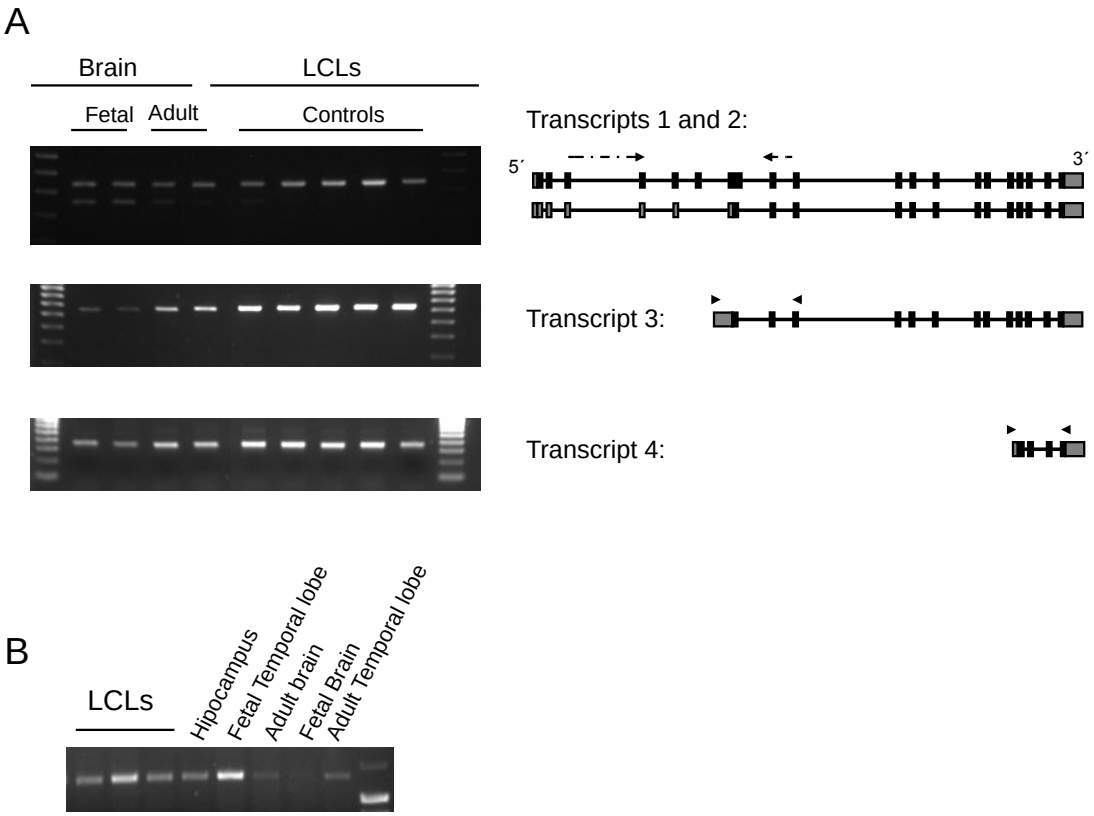

Figure S2. Expression of NSUN2 in Human Tissues

(A) RT-PCRs using transcript-specific primers (locations indicated by arrows in the schematic representations of the transcripts) show the presence of transcripts 1, 3 and 4 in RNA extracts from fetal and adult brain as well as lymphoblastoid cells (LCL). Transcript 2 could only be amplified from fetal brain RNA.

(B) RT-PCRs using primers specific for transcript 1 and 2 show the presence of transcript 1 in all tested tissues.

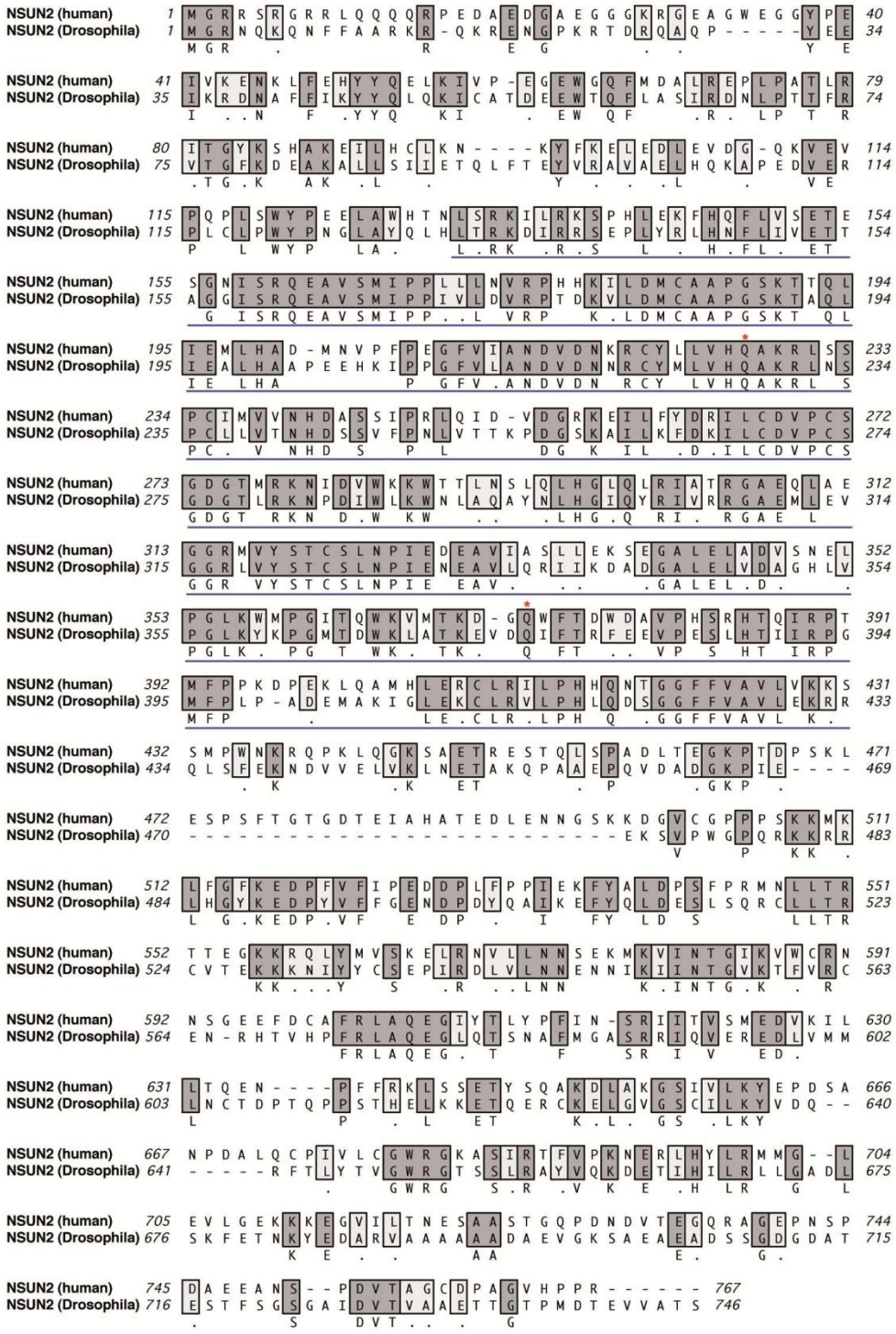

Figure S3. ClustalW Analyses of Human and *Drosophila* NSUN2

ClustalW alignment of human and *Drosophila* NSUN2. Boxed are identical (dark grey) and similar (light grey) amino acids. Blue underlined is the region of about 300 amino acids in the center of the two proteins which shows 74% of similar and 59% of identical amino acids. Asterisks highlight the two nonsense mutations found in affected individuals (p.Gln227* and p.Gln372*).

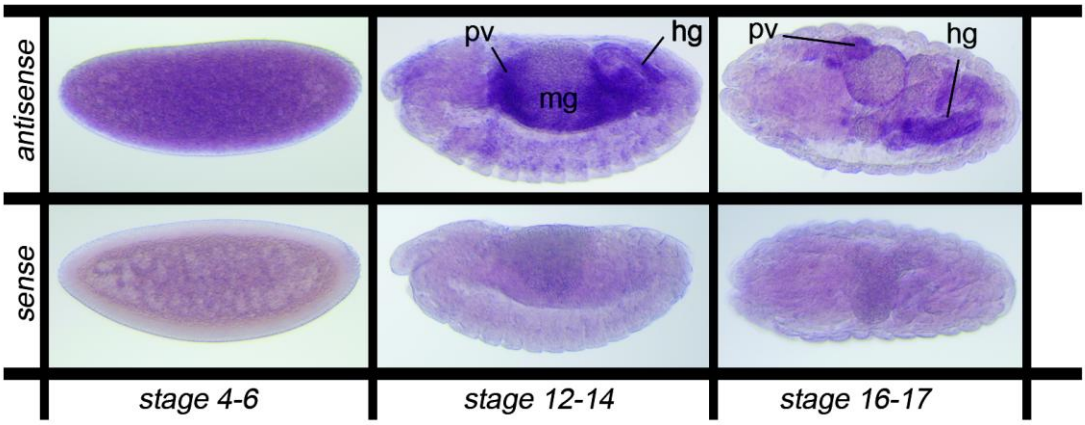

Figure S4. Expression of Drosophila Nsun2 mRNA with In Situ Hybridization

Dioxigenin-labeled sense and antisense probes for dNsun2 were used to visualize dNsun2 mRNA in wild-type embryos (strain w1118). dNsun2 mRNA is expressed with a maternal contribution (stage 4-6, left panel). Older embryos show a weak ubiquitous expression and distinct expression in the proventriculus area (pv) of the foregut and in the hindgut (hg) (stages 12-17). Sense controls (lower panel) stained under the same conditions as antisense (upper panel). Embryos are oriented anterior to the left. mg, midgut.

Table S1. Aversive Olfactory Avoidance and Shock Reactivity

	Olfactory Avoidance		Shock Reactivity
	OCT	MCH	
Canton-S	48.70 ± 2.47	51.52 ± 4.09	53.52 ± 4.32
w^{1118}	47.77 ± 5.66	46.03 ± 3.50	52.69 ± 5.70
$dNsun2^{ex1}$	51.61 ± 3.38	51.26 ± 5.16	54.83 ± 4.55

Male flies were tested for their innate odor and shock avoidance, which also involves elementary locomotion. Denoted are the means of the performance indices ± SEM, ($n \geq 8$).